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Potential applications for cell regulatory factors in liver progenitor cell therapy

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Abstract

Orthotopic liver transplant represent the state of the art treatment for terminal liver pathologies such as cirrhosis in adults and hemochromatosis in neonates. A limited supply of transplantable organs in relationship to the demand means that many patients will succumb to disease before an organ becomes available. One promising alternative to liver transplant is therapy based on the transplant of liver progenitor cells. These cells may be derived from the patient, expanded in vitro, and transplanted back to the diseased liver. Inborn metabolic disorders represent the most attractive target for liver progenitor cell therapy, as many of these disorders may be corrected by repopulation of only a portion of the liver by healthy cells. Another potential application for liver progenitor cell therapy is the seeding of bio-artificial liver matrix. These ex vivo bioreactors may someday be used to bridge critically ill patients to other treatments. Conferring a selective growth advantage to the progenitor cell population remains an obstacle to therapy development. Understanding the molecular signaling mechanisms and micro-environmental cues that govern liver progenitor cell phenotype may someday lead to strategies for providing this selective growth advantage. The discovery of a population of cells within the bone marrow possessing the ability to differentiate into hepatocytes may provide an easily accessible source of cells for liver therapies.

Keywords

Liver progenitor cell; cell therapy; inborn metabolic disorder; bio-artificial liver

Introduction

The liver handles a myriad of essential roles in sustaining vertebrate life. These include the detoxification of foreign compounds, providing an energy reservoir in the form of glycogen, production of bile for the digestion of lipids, and synthesizing a host of key plasma proteins. The liver also possesses a remarkable capacity for regeneration. The mass of the liver in relationship to the mass of the host organism is tightly controlled by the opposing processes of apoptosis and proliferation of mature hepatocytes (Bullough, 1965; Kawasaki et al., 1992;

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Michalopoulos and DeFrances, 1997; Fausto, 2000; Michalopoulos 2010). It is likely that the regenerative properties of the liver evolved as a result of the robust metabolic capacity of the liver. Detoxification of foreign toxins by the liver usually requires two steps. The first step involves activation of the native molecule by mixed function oxidases (i.e. P450s). (Kensler et al.,2002) This step is generally followed by transferase mediated conjugation that results in a polar product which may be excreted. Unfortunately, the intermediate metabolite that exists between these two steps is often an electrophilic molecule that may be several folds more toxic than the parent molecule. (Kensler et al.,2002) Unbalancing of the activation and detoxification steps or depletion of conjugation substrates can result in the accumulation of these toxic intermediates specifically within the liver. The ability to scavenge and adapt to any available food source can provide a tremendous survival advantage. The remarkable regenerative capacity of the liver perhaps evolved to allow sampling of a wide range of foods while allowing recovery of the liver should something toxic be ingested.

Liver regeneration

Liver regeneration, takes place in response to mild to severe liver injury resulting either from physical damage to a portion of the liver or exposure to destructive agents such as hepato-toxins or hepatotropic viruses. Under normal conditions, hepatocytes exhibit minimal replicative activity; only 1 in every 20,000 hepatocytes is undergoing mitotic division at any given time. (Steer, 1995) In the rat, hepatocytes move from the G₀ resting phase of the cell cycle into G₁ within 15 hours of a 70% partial hepatectomy (PH). (Michalopoulos, 2010; Michalopoulos and DeFrances, 1997; Bucher, 1963; Higgins and Anderson, 1931) Peri-portal hepatocytes are the first to undergo DNA synthesis, and proliferation gradually spreads to include hepatocytes located peripheral to the central vein. (Rabes, 1976; Steer, 1995; Michalopoulos and DeFrances, 1997) A large peak of DNA synthesis is observed at approximately 24-hrs post PH and a second, smaller peak occurs at 48 hrs. (Michalopoulos and DeFrances, 1997; Fausto, 2000; Michalopoulos, 2010) The smaller peak reflects DNA synthesis associated with the proliferation of non-parenchymal cells (NPC) and peri-central hepatocytes. Unlike hepatocytes, which exhibit a progressive involvement of DNA synthesis from the peri-portal zone to the peri-central zone, NPCs exhibit simultaneous DNA synthesis across the liver lobule. (Bucher, 1963) The original liver mass is restored within 10 days. (Higgins and Anderson, 1931 Duncan, 2009)

Several animal models of chemical hepatotoxicity have been developed to study the mechanisms regulating the proliferative response to liver injury. Among the most extensively utilized chemical agents in the rat are carbon tetrachloride (CCl₄), which causes necrosis of the centrilobular regions of the liver, and allyl alcohol (AA), which causes peri-portal necrosis. (Edwards et al.,1993; Reid, 1972; Badr, 1986; Lombardi and Ugazio, 1965) In both models, regeneration of the necrotic region is mediated primarily by proliferation of mature hepatocytes, though the AA model can have a significant progenitor cell component depending on the specific conditions of the protocol.

Liver progenitor cells

Progenitor cells in the liver represent an alternative source of cells for liver regeneration. (Fausto and Campbell, 2003) A majority of the data on liver progenitor cells has been generated in experimental rodent models. In the rodent, liver progenitor cells are referred to as “oval cells”. These cells are bipotential, capable of giving rise to both hepatocytes and cholangiocytes. Morphologically, oval cells are small in size (approximately 10 μm in diameter), with a large nuclear to cytoplasm ratio, and an oval shaped nucleus (hence their name). (Fausto, 1994) Proliferating oval cells in both the rat and mouse models appear to

radiate from the peri-portal region, forming primitive ductular structures with poorly defined lumen. (Evarts et al., 1987; Lee et al., 1997) They are similar to bile ductular epithelial cells in their distinct immunophenotypic profile, expressing certain keratin markers (e.g. CK19), and gamma-glutamyl transpeptidase (GGT). (Petersen et al., 1998) Oval cells may also express high levels of alpha-fetoprotein (AFP). (Sell, 1981) Monoclonal antibodies such as OV1, OV6, OC.2 and BD1 in the rat, and A6 in mice, have been developed to aid in their identification. (Dunsford et al. 1989; Engelhardt et.al., 1990; Hixson et al., 1990; Faris et al., 1991; Yang et al. 1993)

In recent years, oval cells have come to be understood as a very heterogeneous population. Depending on the injury model used and the species being studied, bipotential liver progenitor cells may express a myriad of different markers. Some examples of recently identified rat oval cell markers include CD133, Claudin-7 and EpCAM. (Yovchev et.al., 2007) Not surprisingly, these markers are also expressed in bipotential cells within the developing liver. In the mouse, CD24a and Foxl1 have been identified in hepatic oval cells. (Ochsner et.al., 2007; Sackett et.al., 2009) It is not yet known whether these new markers are species specific.

The origin of oval cells remains unclear. Due to their involvement in peri-portal repair, some believe they exist in very small numbers within the peri-portal region of the liver lobule, and that they emerge from that niche in response to injury. (Brill et al., 1993; Golding et al., 1996; Kuwahara et.al., 2008) Though oval cells do not normally participate in the regenerative response to PH or CCl₄ injury, they can be forced to do so by suppressing mature hepatocyte proliferation. Administration of N-2-acetylaminofluorene (2AAF), prior to, and during hepatic injury induced by PH or CCl₄, will block the proliferation of hepatocytes by interfering with the cyclin D1 pathway. Oval cell proliferation can thus be induced in these otherwise non-oval cell-aided regeneration models. (Evarts et al., 1990; Fausto et al., 1992; Fausto and Campbell, 2003) As with AA induced injury, oval cell proliferation in these models begins in the peri-portal region before arborizing into the mid-zone as regeneration proceeds. While the 2AAF/PH model for oval cell activation has arguably become the predominant rat model, several alternative models are favored by certain laboratories (i.e. choline deficient/ethionine supplemented diet in rat and mice, 3,5-diethoxycarbonyl-1,4-dihydrocollidine in mice).

It has been reported that in an injury model where the bile duct epithelium was destroyed prior to oval cell activation, the oval cell response was severely inhibited. (Petersen et al., 1997) These studies support the idea that oval cells arise from somewhere within the biliary tree, likely from the Canals of Hering. (Grisham and Thorgeirsson, 1997) However, it has also shown a percentage of oval cells may actually arise from an extra-hepatic source, within the bone marrow. (Petersen et al., 1999; Theise et al., 2000a; Oh et al., 2007) Several other investigators have provided evidence that BM-derived cells possess the ability to produce functional hepatocytes and bile ductular cells. (Theise et al., 2000b; Lagasse et al., 2000; Alison et al., 2000) Other studies have demonstrated little or no contribution of bone marrow derived epithelial cells to liver regeneration. (Wagers et. al., 2002; Menthena et. al., 2004; Rountree et. al., 2007) It is important to note, however, that two of these studies did identify a very low level of BM contribution which, when extrapolated to the entire liver, represents over one hundred thousand bone marrow derived cells.

In human liver, progenitor cells are simply referred to as hepatic progenitor cells. While data on these cells are much more limited than data from experimental rodent models, there is evidence that progenitor cells are induced to proliferate in several liver pathologies including viral hepatitis and cirrhosis. (Xiao, et al., 2004; Libbrecht et al., 2000) Additionally, several inborn metabolic disorders result in chronic liver diseases that involve

the proliferation of hepatic progenitor cells. (Lowe et al., 1999) These are chronic injuries that may not be well represented by the acute injury models employed for the induction of oval cells in experimental animal models. Despite this potential shortcoming, there is no reason to believe that bipotential liver progenitor cells in humans represent a drastically different cell phenotype than the oval cells that are studied in laboratories around the world. It has been the focus of our laboratory, as well as several others, to harness the regenerative capabilities of hepatic progenitor cells for the treatment of human liver diseases. The finding that liver progenitor cells may be obtained from easily accessible location (bone marrow) significantly enhances their therapeutic potential.

Mature hepatocytes for liver therapies

Mature hepatocytes have been used in clinical trials for the treatment of hereditary hyperbilirubinemia. (Fox et al., 1998) Despite the transplant of number of cells equivalent to approximately 2.5% of the recipient liver mass, only a modest improvement in bilirubin metabolism was noted. Additionally, this treatment did not alleviate the eventual need for a liver transplant. (Ito et al., 2009) This outcome was expected, as the population of transplanted cells was under no pressure to expand in vivo. Experiments in animal models have demonstrated that even following a PH, expansion of the transplanted cell population is insufficient to cure most diseases. (Laconi et al., 1999) Experimental animal models that provide a survival or proliferation advantage to the transplanted cells have yielded much more promising results.

A mouse model for hereditary tyrosinemia type 1 does provide such an advantage to the transplanted hepatocyte population. These mice do not express fumaryl acetoacetate hydrolase (FAH), and they build up toxic levels of an intermediary tyrosine metabolite within the hepatocytes population. The toxic effect of this metabolite can be mitigated by treatment with the drug 2(2-nitro-4 trifluoromethylbenzoyl)-1,3 cyclohexane dione (NTBC). Transplanting hepatocytes that possess normal tyrosine metabolic capability into the livers of these animals, followed by withdrawal of NTBC treatment resulted in a robust expansion of the transplanted cell population. (Overturf et al., 1998) These two results highlight both the potential of hepatocyte transplant in situations where the transplant has a survival or growth advantage, as well as the limitations in situations where no survival advantage exists.

Advantages of using progenitor cells for liver therapies

There are several potential advantages to using progenitor cells rather than mature hepatocytes for cell based liver therapies. The first of these is the virtually unlimited regenerative capacity of the progenitor cell population. Mature hepatocytes do retain the ability to proliferate in their terminally differentiated state. (Overturf et al., 1997) However, progenitor cells are able to proliferate indefinitely and are also able to migrate through areas of injury much more effectively than differentiated hepatocytes. Another advantage may be found in the bipotential nature of progenitor cells. These cells have the potential to regenerate both the hepatic cords as well as the biliary tree. It is also possible that rare, true liver stem cells may be isolated along with the transient amplifying population of progenitor cells. These asymmetrically dividing cells may become integrated into the recipient liver, providing an inexhaustible source of cells for continuous regeneration.

Perhaps the strongest argument that can be made for using progenitor cells for liver therapies is the potential for transplanting autologous cells. The discovery that bone marrow may give rise to liver cells has opened the possibility that cells may be obtained from bone marrow aspirates, expanded and treated in vitro, and transplanted back into the patient from which they were obtained. (Petersen et al., 1999) This would alleviate the need for life-long

immunosuppression required for the maintenance of both whole liver transplant as well as the transplant of mature hepatocytes isolated from cadaveric liver.

Potential applications of liver progenitor cell therapies

Regeneration of liver parenchyma lost to disease may be aided by liver progenitor cells transplant into the region of injury. The obvious problem with this application is that once the transplanted progenitor cells engraft and differentiate, they would likely come under the influence of whatever pathology brought about the need for medical intervention. There may be certain chronic conditions where repopulation of the liver resident progenitor cell population may bolster the regenerative capacity of the liver, though these situations would be quite rare. Another potential use would be to aid the recovery of the liver following acute toxicity brought about by exposure to a chemical. Acetaminophen intoxication represents the most clinically relevant example of acute liver injury. (Larson et al., 2005) However, the time that would be required for cell collection, transplantation, engraftment, proliferation and differentiation would seem to limit the practicality of this potential application.

The most promising application of progenitor cell therapy in the liver would be for the correction of inborn metabolic disorders (IMD). IMDs represent a collection of disorders that result from mutation or deletion of a gene that encodes a protein required for one of the liver's critical functions. (Arroyo and Crawford, 2006) Monogenic disorders are generally inherited in an autosomal recessive manner, and result from a single point mutation. (Aaseth et al., 2007; Scaglia and Longo, 1999; Latta and Brodehl, 1990) Parents of an afflicted child each carry a single copy of the mutant gene, conferring a 25% chance that each child will be born with the disease. IMDs are rare individually, but combined may account for up to 20% of non-chromosomal genetic disorders in full term births. It has been estimated that the incidence of IMDs is approximately 1:5,000. (Ward, 1990) Ex vivo transduction of progenitor cells with a healthy copy of the missing or mutated gene, followed by transplant back to the neonatal liver represents a possible strategy for curing inborn metabolic disorders.

Another promising application for liver progenitor cells does not involve the direct treatment of patients, but instead may be used indirectly, as material for the population of a bio-artificial liver (BAL). These are closed extracorporeal systems containing a matrix, on which, liver cells may be grown. (Zeilinger et al., 2004; Riccalton-Banks et al., 2003; Gomez-Lechon et al., 1998) Once populated with functional liver cells, the BAL would be placed in-line with the patient's circulatory system (ex vivo.) The purpose of BALs would be to sustain the life of a patient whose liver has failed until a transplantable organ becomes available. Thus far, the population of BAL by mature liver cells has met with limited success. (Zeilinger et al., 2004; Riccalton-Banks et al., 2003; Gomez-Lechon et al., 1998; Linke et al., 2007) Long term maintenance of mature hepatocytes within BAL has proven to be a challenge. It is possible that BAL seeded by progenitor cells may retain a population of undifferentiated cells that could proliferate to colonize areas of BAL that contain hepatocytes that have died. It is also possible that BAL may benefit from the migratory property of liver progenitor cells, allowing for uniform distribution of cells within the matrix of the BAL.

Liver progenitor cells may prove useful in the development of bioengineered livers. (Shupe et al., 2010; Uygun et al., 2010) Similar to BAL, these engineered organs are composed of a natural or artificial matrix that is colonized by liver cells. Unlike the extracorporeal BAL intended to bridge patients to another therapy, bioengineered livers are transplanted into the patient as a substitute for a cadaveric liver. Progenitor cells would seem to be the most obvious candidate cell for colonizing these organs, as their bipotential phenotype would

allow for establishment of both functional hepatocytes and the biliary tree. It may also be possible to mimic, to some degree, stem cell mediated liver regeneration within these organs by using a series of media supplemented with growth factors that regulate liver progenitor cell behavior. This might allow controlled transition from a highly proliferative/migratory phenotype that would benefit colonization of the organ to a differentiation phenotype that would result in functional tissue.

Regulation of progenitor cell phenotype

The major obstacle to the development of liver progenitor cell therapies is providing some form of an advantage to the transplanted cells. In situations where the disease to be treated causes the destruction of native cells, this growth advantage is intrinsic to the transplant. However, in a majority of inborn metabolic disorders, the effects of the disease are systemic. An example of this would be Crigler-Najjar syndrome. This is a monogenic disorder in which one of the enzymes responsible for bilirubin metabolism is missing. While the patient suffers from hyperbilirubinemia, there is no specific consequence to the hepatocytes that lack the gene. While transplanting progenitor cells into the livers of these patients would most certainly transfer some degree of bilirubin metabolism capability, the level of this capability will be dictated by the level of cell engraftment. Much of the promise inherent to progenitor cell based therapies lies in the potential for continued expansion and repopulation of the liver by healthy cells. Without a survival or growth advantage, many of the potential advantages of these therapies are lost.

Expansion of a progenitor cell transplant could be accomplished by chemical, radiological or surgical damage to the recipient organ. This strategy is appealing neither from a practical, nor an ethical viewpoint. A much more appealing approach would be to treat the progenitor cells, prior to transplant, or the patient, following transplant, in such a manner as to create an environment within the liver that is conducive to progenitor cell proliferation. Of these two approaches, the former would be preferred, as systemic treatment with growth factors may have undesirable consequences in organs other than the liver.

The need for a means to control liver progenitor cell phenotype has led many laboratories, including our own, to study the molecular signaling microenvironment in which liver progenitor cells proliferate and differentiate. Several growth factors that regulate progenitor cell phenotype have been known for some time. These factors include hepatocytes growth factor, fibroblast growth factors 1 and 2, stem cell factor and epidermal growth factor. (Isfort et al., 1998) Each of these factors has been validated through extensive study, and each is required for maximal progenitor cell proliferation in experimental rat models. In the case of HGF, studies have shown that systemic administration of the factor can influence the progenitor cell response to 2AAF within the liver. (Alison et al., 1993; Nagy et al., 1996) Recently, a host of other regulatory factors have been identified. Examples of these would include: interferon gamma, tumor necrosis factor, interleukin-6 and TNF-like weak inducer of apoptosis. (Bisgaard, et al., 1999; Knight et al., 2000; Matthews et al., 2004; Jakubowski et al., 2005) Each of these factors influences at least one aspect of the liver progenitor cell phenotype such as migration, proliferation or differentiation, and may be incorporated into therapies as a means to maximize the level of organ repopulation.

Over the past decade, our own laboratory has strived to contribute to our understanding of the molecular regulation of liver progenitor cell phenotype. The following represents a handful of regulatory factors that we have identified along with some examples of how this knowledge may be useful in the development of liver progenitor cell based therapies.

Connective Tissue Growth Factor (CTGF)

CTGF is a secretory protein and a member of the CCN protein family. (Bork, 2009; Moussad and Brigstock, 2000) This factor has the ability to modulate several aspects of cell phenotype including proliferation, apoptosis, differentiation, angiogenesis, migration, adhesion, and ECM production. (Moussad and Brigstock, 2000) In the liver, CTGF is known primarily for its ability to mediate fibrotic processes, including both the activation and proliferation of fibroblasts. (Rachfal and Brigstock, 2003; Song et al., 2007) In normal livers, CTGF is expressed at a low level by venous endothelial cells and arterial myocytes. (Sedlacek et al., 2001) However, a significant induction of CTGF transcription is induced following experimental injury to the liver. (Ujike et al., 2000; Pi et al., 2005, 2008) Immunohistochemistry and in situ hybridization indicate the expression of CTGF by several types of cells within the liver, including stellate cells and proliferating epithelial cells. (Sedlacek et al., 2001; Ujike et al., 2000; Paradis et al., 1999)

The prostacyclin derivative, Iloprost, can effectively suppress the induction of CTGF synthesis following 2AAF/PH in the rat. (Pi et al., 2005; Stratton et al., 2002) Repression of CTGF expression by iloprost in the 2AAF/PH model results in a significantly blunted progenitor cell reaction. (Pi et al., 2005) Further studies have indicated that CTGF will bind to fibronectin. This fact may be relevant for two reasons. First, degradation of the extracellular matrix within the damaged regions of the liver may liberate bound CTGF, and increase the levels of serum CTGF. The second reason is that during progenitor cell mediated regeneration in rats; a fibronectin rich provisional matrix is secreted within the portal zones of the liver. This would result in a concentration of the factor within the areas that progenitor cells are proliferating.

Application for therapy

Further understanding of how deposition of the fibronectin rich provisional matrix is regulated may lead to a strategy for increasing the fibronectin component of the livers of patients preparing for liver stem cell therapy. This may provide a microenvironment that favors the expansion of transplanted progenitor cells within these livers. One potential way to accomplish this would be to transplant a combination of progenitor cells along with a fibroblast population that had been previously conditioned to secrete the fibronectin rich matrix. It is possible that the fibroblast component of the transplant could affect limited remodeling of the matrix within the diseased liver. This may, in turn, increase the local concentration of CTGF within areas in which progenitor cells engraft, and help maintain a high proliferation rate in the transplanted population.

Studies on CTGF and fibronectin may also be applied to the construction of BAL matrices. A fibronectin rich matrix seeded with progenitor cells and fibroblasts would concentrate CTGF on the matrix and provide an improved microenvironment for the proliferation of undifferentiated cells. Triggering fibroblast remodeling to the collagen rich matrix may promote differentiation of the progenitor cell population resulting in a well populated system.

Insulin-like growth factor binding protein 3 (IGFBP3)

CD34⁺ stem cells will migrate towards higher concentrations of IGFBP3 in a mouse model for retinopathy. In addition, IGFBP3 is able to induce the differentiation of CD34⁺ cells down the endothelial lineage. Exogenous IGFBP-3 as well as transgenic over expression of IGFBP3 protects against vascular damage and promotes a higher rate of progenitor cell mediated vascular repair in the retinopathy model. (Chang et al., 2007) Other studies have shown that exogenous IGFBP-3 will induce proliferation of undifferentiated hematopoietic

cells (CD34⁺, CD38⁻) (Liu et al., 2003) It has also been reported that IGFBP3 levels are increased during liver regeneration following 2AAF/PH in rats, and hepatic progenitor cells will migrate in response to an IGFBP3 gradient in vitro. (Under revision: The Journal of Hepatology) This is not surprising given the recently discovered phenotypic similarities between hematopoietic stem cells and liver progenitor cells. (Petersen et al., 1998) It appears that IGFBP3 functions as a chemotactic factor during progenitor cell mediated liver regeneration. It is worth noting that this affect is independent of any association with insulin-like growth factor.

Somatostatin (SST)

SST is a unique regulatory hormone, which was first reported as a hypothalamic hormone that can inhibit growth hormone secretion. (Brazeau et al., 1973) SST has two biologically active forms and is upregulated in the liver during progenitor cell mediated liver regeneration. (Jung et al., 2006) The major actions of this hormone include the regulation of hormone secretion by the pituitary gland and pancreas, as well as suppression of secretory activity in other endocrine and exocrine organs. (Bertherat et al., 1995) SST has also been shown to control the proliferation of both normal and tumor cells. (Weckbecker et al., 2003) In addition, SST is involved in the differentiation and migration of thymocyte precursor cells. (Solomou et al., 2002) These broad biological functions are all communicated through five receptor subtypes, all of which are G-protein-coupled receptors. SST receptors (SSTRs) are widely distributed throughout many tissues, and the effects of SST binding to these receptors are dependent on the phenotype of the cell expressing the receptor. (Weckbecker et al., 2003; Lahlou et al., 2004) It has been recently discovered that SST may act as a strong chemotactic factor during liver progenitor cell migration within the regenerating liver. In vitro studies performed in trans-well migration chambers indicate that nanomolar concentrations of SST are able to increase progenitor cell migration by up to 3.5 fold. (Jung et al., 2006) SSTR4 was identified on the surface of rat liver progenitor cells, and SST concentrations within the liver are drastically increased following 2AAF/PH. (Jung et al., 2006) The increase in SST peaks on day 11 in the 2AAF/PH model, the timepoint when maximum progenitor cell proliferation is usually seen.

Application for therapy

The use of chemotactic factors such as IGFBP3 or SST in liver progenitor cell therapy would likely involve increasing engraftment of transplanted cells into the recipient liver. To be an effective component of a progenitor cell based therapy, it may be necessary to increase the expression of the factor within the liver. This would require preliminary treatment of the patient to induce expression. The molecular signals that regulate SST and IGFBP3 expression within the liver have yet to be determined, rendering this strategy quite hypothetical. It is, however, possible that infusion of a chemotactic factor into the peripheral circulation could positively influence migration of progenitor cells within the liver. This could result in greater infiltration of the organ by the proliferating transplanted cell population.

A much more immediate application for these factors would be in the population of a bio-artificial liver. It is difficult to administer cells to areas deep within an artificial matrix. Cells expressing, or material impregnated with, these factors may be incorporated into the system's matrix. This could facilitate the migration of seeded progenitor cells throughout the matrix and result in more uniform population of the system.

Stromal cell derived factor 1 (SDF-1)

Several lines of evidence exist suggest that CXC chemokines are critical participants in generalized wound healing responses. First, elevated levels of many CXC chemokines and their receptors are expressed in injured tissue, including the liver. (Mavrier et al., 2004) Second, CXC chemokines have potential mitogenic effects on several tissue-resident cells including epithelial, endothelial and inflammatory cells. (Mavrier et al., 2004; Avniel et al., 2006; Butts et al., 2003) Third, many of the previously mentioned cells begin to express increased levels of CXC chemokines following inflammatory stimuli. For example, hepatocytes and hepatic stellate cells have been shown to express CXC chemokines following exposure to acetaminophen and other hepatotoxic agents. (Ren et al., 2003) Taken together, these findings indicate that CXC chemokines, in general, may play an instrumental role in several forms of tissue regeneration. One specific CXC chemokine, SDF-1, appears to play a central role in the regulation of liver progenitor cell phenotype.

Several research groups have reported direct evidence for SDF-1 cytokine participation in the recruitment of BM-derived progenitor cells to the injured liver. (Jin et al., 2009; Lei et al., 2009; Kucia et al., 2008; Hatch et al., 2002) Upregulation of SDF-1 during the the progenitor cell response to 2AAF/PH injury in the rat has also been documented. (Hatch et al., 2002; Zheng et al, 2006) Conversely, there is no up-regulation of SDF-1 during non-progenitor cell mediated liver regeneration. (Zheng et al, 2006) Liver progenitor cells have also been shown to express the only known receptor for SDF-1 (CXCR4). (Hatch et al., 2002) Liver progenitor cells migrate in response to an SDF-1 concentration gradient in culture, and SDF-1 mobilizes cells within the bone marrow that express the embryonic liver protein, alpha fetoprotein. (Kucia et al., 2008; Hatch et al., 2002) These data support the notion that SDF-1 may be of significant value in the development of liver therapies based on bone marrow derived liver progenitor cells.

Granulocyte-Colony Stimulating Factor (G-CSF)

Granulocyte-colony stimulating factor is a cytokine that is produced by a variety of cell types. It has been shown to regulate both proliferation and differentiation of granulocytes precursor cells, and mediates the mobilization of hematopoietic stem cell to peripheral blood. (Gordon et al., 2006) Recent studies clearly indicate that G-CSF may be effective in activating a cell population within the bone marrow that may contribute to liver regeneration following injury. (Yannaki et al., 2006; Quintana-Bustamante et al., 2006; Huiling et al., 2004; Liu et al., 2006; Gaia et al., 2006) G-CSF treatment during liver regeneration following injury can both speed recovery, and increase the contribution of bone marrow derived cells participating in the process. (Liu et al., 2006; Piscaglia et al., 2007) G-CSF has been used to treat humans with cirrhosis, and some clinically relevant improvement has been reported. (Yannaki et al., Pardo et al., 1995; Gurakar et al., 1994) These studies have also noted increased recruitment of bone marrow derived cells to the diseased liver. Studies in rats have confirmed that G-CSF does, in fact, increase both the endogenous progenitor cell response as well as the contribution of bone marrow derived epithelial cells to liver regeneration following 2AAF/PH. (Piscaglia et al., 2007)

Application for therapy

SDF-1 and G-CSF both appear to increase the mobilization of bone marrow cells to the liver. We are currently exploring the possibility that these factors may be incorporated into an indirect progenitor cell therapy for the treatment of inborn metabolic disorders. Our current vision of such a therapy involves the isolation of a cell population with hepatocyte potential from a patient. These cells would be transduced, in vitro, with a healthy copy of the mis-coded gene. These cells would then be transplanted back into the patient where they

would re-incorporate into the bone marrow. The necessity for myoablation would presumably be dictated by the level of bone marrow repopulation required. Treatment with factors such as SDF-1 and G-CSF would be used to increase trafficking of transplant derived cells to the liver. It remains to be seen if, over time, a treatment like this could result in a degree of liver repopulation sufficient to cure disease. There is some evidence that it would. We are aware of at least one instance in which an individual with glycogen storage disease type-1a underwent a bone marrow transplant for an unrelated condition. Following the transplant, the metabolic disorder improved. (Personal communication: Dr David Weinstein; Shands Hospital; Gainesville, FL; manuscript in preparation)

Transforming growth factor β (TGF β)

It is possible that several signaling factors that modulate liver progenitor cell phenotype may be under the control of a common transcription factor. TGF β is a well characterized regulator of epithelial cell phenotype. (Matsuzaki, 2009) Liver progenitor cells are much more resistant than hepatocytes to the pro-apoptotic effects of TGF β . (Ding et al., 2009) It has also been determined that that TGF β mediates the upregulation of CTGF in portal fibroblasts. (Pi et al., 2005) IGFBP3 is known to directly bind the TGF β receptor. (Kuemmerle et al., 2004) It has also been demonstrated that IGFBP3 potentiates TGF β signaling by increasing the phosphorylation of Smad2 and Smad3 in the presence of TGF β . (Fanayan et al., 2002) This can have the effect of increasing responsiveness of receptor positive cells to TGF β . TGF β receptor is present on both the mouse liver progenitor cell membrane (personal communication from Dr Bart Rountree; Hershey Medical Center; Hershey, Pa.) as well as the stellate cell membrane. Other studies have indicated that TGF β may play a role in regulating SST expression. (Leu et al., 2008) This suggests that TGF β is a key modulator of several of the liver progenitor cell regulation factors discussed in this review.

Application for therapy

It is possible that treatment of a patient undergoing liver stem cell therapy with low doses of TGF β may augment several aspects of the therapy. In this example, TGF β may confer a growth advantage to the transplant by inducing a small degree of apoptosis within the hepatocyte population will still allowing expansion of the progenitor cell transplant. This would occur coincidently with the augmentation of CTGF, IGFBP3 and SST activities as described above.

Conclusion

While effective therapies involving liver progenitor cells remain a distant goal, the works of several laboratories are currently laying the groundwork in the present. Monogenic inherited metabolic disorders represent a reasonable first goal for such treatments. The development of bio-artificial livers may also benefit from these studies.

Abbreviations used in this paper

PH	partial hepatectomy
2AAF	N-2-acetylaminofluorene
NPC	non-parenchymal cells
CCl₄	carbon tetrachloride
AA	allyl alcohol

AFP	alpha fetoprotein
NTBC	2(2-nitro-4 trifluoromethylbenzoyl)-1,3 cyclohexane dione
IMD	inborn metabolic disorder
BAL	bio-artifical liver
CTGF	connective tissue growth factor
IGFBP3	Insulin-like growth factor binding protein 3
SST	somatostatin
SDF-1	Stromal cell derived factor 1
GCSF	Granulocyte-Colony Stimulating Factor
TGFβ	Transforming growth factor β
P450	Cytochrome P450
CK19	Cytokeratin 19
GGT	Gamma-glutamyl transpeptidase

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Figure 1. Potential applications of liver progenitor cell regulatory factors in cell therapy

1) Progenitor cells are isolated either from the patient's own liver or bone marrow. G-CSF and SDF-1 may increase the number of cells within the bone marrow that possess the ability to differentiate into epithelial cells. **2)** Following isolation, the liver progenitor cell population is expanded in culture. Several growth factors, particularly HGF, greatly enhance the survivability and maintenance of the undifferentiated state in culture. For the correction of inborn metabolic disorders, cells may be transfected with a normal copy of the miscoded gene(s). **3)** The cultured cells may be used to seed bio-artificial liver matrix. The combination of a fibronectin rich matrix with CTGF supplemented media may promote more robust colonization of the matrix. It is also possible that material within the matrix impregnated with IGFBP3 or SST would promote migration of the progenitor cells throughout the matrix. **4)** Cells may be transplanted back into the liver through the organ's circulation. Pretreatment of the cells or systemic treatment of the patient with chemotactic factors such as SST and IGFBP3 may promote migration of the cells within the organ. Additionally, simultaneous transplant of fibroblasts conditioned to produce the fibronectin rich provisional matrix may provide an environment that promotes progenitor cell migration and proliferation. **5)** It may also be possible to transplant curative progenitor cells into the bone marrow. **6)** Mobilization of these cells from the bone marrow to the liver may be positively influenced by G-CSF and SDF-1.